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134 (2)

No.

In the Supreme Court of the United States

APOTEX, INC. and APOTEX CORP.,

Petitioners,

v.

SANOFI-SYNTHELABO, SANOFI-SYNTHELABO INC., and BRISTOL-MYERS SQUIBB SANOFI PHARMACEUTICALS HOLDING PARTNERSHIP,

Respondents.

On Petition for a Writ of Certiorari to the United States Court of Appeals for the Federal Circuit

SUPPLEMENTAL APPENDIX

ROBERT B. BREISBLATT
Katten Muchin Rosenman LLP
525 W. Monroe Street
Chicago, IL 60661
(312) 902-5200

ROBERT S. SILVER
Caesar, Rivise, Bernstein, Cohen & Pokotilow, Ltd.
1635 Market Street 11th Floor
Philadelphia, PA 19103
(215) 567-2010

ROY T. ENGLERT, JR.
Counsel of record
ALAN E. UNTEREINER
DANIEL R. WALFISH
Robbins, Russell, Englert, Orseck, Untereiner & Sauber LLP
1801 K Street, N.W. Suite 411
Washington, D.C. 20006
(202) 775-4500

SHASHANK UPADHYE
Apotex, Inc.
150 Signet Drive
Toronto, ON M9L 1T9
Canada
(416) 401-7701

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Pursuant to Supreme Court Rule 33(1)(c), petitioners have separately duplicated certain patent documents on 8½- by 11-inch paper in this Supplemental Appendix.

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U.S. Patent No. 4,847,265 ..... S29

United States Patent [19]

Aubert et al.

[11] Patent Number: 4,529,596

[45] Date of Patent: Jul. 16, 1985

[54] THIENO [3,2-c] PYRIDINE DERIVATIVES AND THEIR THERAPEUTIC APPLICATION

[75] Inventors: Daniel Aubert, Plaisance Du Touch; Claude Ferrand, Ramonville Saint-Agne; Jean-Pierre Maffrand, Garonne, all of France

[73] Assignee: Sanofi, S.A., Toulouse, France

[21] Appl. No.: 510,582

[22] Filed: Jul. 5, 1983

[30] Foreign Application Priority Data

Jul. 13, 1982 [FR] France 82 12599

[51] Int. Cl.³ A61K 31/435; C07D 495/04

[52] U.S. Cl. 514/231; 514/301; 544/127; 544/362; 546/114

[58] Field of Search 546/114; 544/362, 127; 424/248.51, 250, 256

[56] References Cited

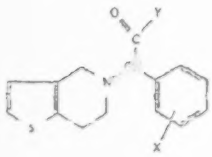
U.S. PATENT DOCUMENTS

2,527,574 10/1950 Ruevschi, Jr. 546/138

Primary Examiner—Henry R. Jiles
Assistant Examiner—Bernard L. Dentz
Attorney, Agent, or Firm—Weiser & Stapler

[57] ABSTRACT

This invention relates to new thieno [3,2-c] pyridine derivatives having the formula:



(I)

in which:

Y represents the OH group or an OR group in which R is a straight or branched lower alkyl radical, or Y represents a group

in which R_1 and R_2 are each independently of each other, hydrogen or a straight or branched lower alkyl group, or R_1 and R_2 form together and with the nitrogen atom to which they are attached a heterocycle which may include a second heteroatom such as oxygen or nitrogen, wherein the latter may be substituted by a lower alkyl or benzyl radical which may be substituted, and X represents hydrogen, a halogen or a lower alkyl radical; and their addition salts with pharmaceutically acceptable mineral or organic acids if Y represents the group OR or

or with mineral bases if Y represents OH, as well as the two enantiomers or their mixture.

The invention also relates to the preparation process of new thieno [3,2-c] pyridine derivatives and to their therapeutic application as blood-platelet aggregation inhibiting agents and antithrombotics.

12 Claims, No Drawings

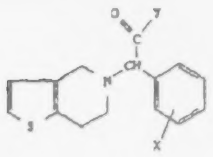
DEPOSITION EXHIBIT Pefts 1 3/26/03 TS

CLO-DRL 51129

S1

THIENO [3,2-c] PYRIDINE DERIVATIVES AND THEIR THERAPEUTIC APPLICATION

This invention relates to new thieno[3,2-c]pyridines, to a process for their preparation and to their therapeutic applications. The new derivatives of this invention have the following general formula:



(I)

in which:

Y represents the OH group or an OR group in which R is a straight or branched lower alkyl radical, or Y represents a group

in which R_1 and R_2 are each independently of each other hydrogen or a straight or branched lower alkyl group, or R_1 and R_2 form together and with the nitrogen to which they are attached a heterocycle which may include a second heteroatom such as oxygen or nitrogen, wherein the latter may be substituted by a lower alkyl or benzyl radical which may be substituted, and X represents hydrogen, a halogen or a lower alkyl radical.

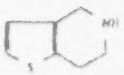
These compounds having an asymmetrical carbon may exist in the form of two enantiomers. The invention relates both to each enantiomer and their mixture.

The invention also includes addition salts with pharmaceutically acceptable mineral or organic acids if Y represents the groups OR or

or with mineral bases if Y represents OH.

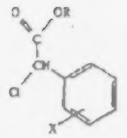
A lower alkyl radical is understood to mean a C_1–C_6 saturated hydrocarbon chain.

The invention also relates to a process for the preparation of compounds of the formula (I) as defined above, wherein the esters of the invention in which Y represents an OR group as defined above are prepared by condensation of 4,5,6,7-tetrahydro-thieno[3,2-c]-pyridine, having the following formula:



(II)

with an α-chlorophenyl acetate having the following formula:



(III)

in which R and X have the above-defined meanings. Acids of formula (I), in which Y represents OH, are obtained by saponification.

To prepare the amides, in which Y represents a group

in which R_1 and R_2 have the above-defined meanings or even some esters of formula (I), the acid of formula (I)(R=OH) is reacted, after activation if required, either with the amine

or with the alcohol R—OH.

The α-halogenated esters of the formula (III) are prepared using known methods (E. L. ELIEL, M. T. FISK and T. PROSSER, Organic Syntheses, Coll. Vol. IV, J. WILEY and SONS, Inc. New York, 1963, p. 169).

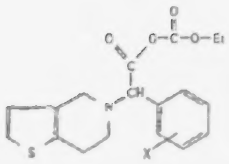
Although it is possible to obtain all the esters of formula (I) by the reaction between the compounds of formulae (II) and (III), for economic reasons it is preferable to prepare certain higher esters of the formula (I) from the acid of formula (I) and alcohol (R—OH).

The condensation of the tetrahydro-thieno-pyridine with the ester of formula (III) is effected in the presence of an alkali metal carbonate, such as potassium carbonate for example, in an inert solvent such as dimethylformamide, tetrahydrofuran or 1,2-dimethoxy ethane, at temperatures between 60° C. and the boiling point of the solvent.

The saponification of the ester of formula (I) in which R is methyl or ethyl is effected by an alkali metal hydroxide such as sodium or potassium hydroxide in a hydroalcoholic solvent, at temperatures between room temperature and the boiling point of the solvent.

Activation of the acid of formula (I) may be obtained by treatment with ethyl chloroformate in the presence of a slight excess of triethylamine at temperatures between −5° C. and 0° C. in an inert solvent such as chloroform, 1,2-dimethoxy ethane or tetrahydrofuran.

A mixed anhydride having the following formula



(IV)

is formed and if treated in situ with a slight excess either of alcohol or amine, at temperatures between 10° C. and room temperature, the esters or amides of the formula (I) will be formed respectively.

The activation of the acid of formula (I) may also be obtained in different ways: the amides of formula (I) were also thus prepared by condensing the acid (I) with the amine

in the presence of dicyclohexylcarbodiimide in solution in 1,2-dichloro-ethane.

The esters of formula (I) may also be obtained in the conventional manner by condensing the corresponding acid and alcohol (ROH) in the presence of hydrochloric gas or thionyl chloride.

The following non-limiting Examples are given to illustrate the invention.

EXAMPLE 1

Methyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetate (R_1 = —CH_3; X = 2-Cl) derivative No. 1.

3.47 g (0.144 mole) of methyl 2-chloro-α.chlorophenylacetate and 19.82 g (0.144 mole) of potassium carbonate are added to a 20 g (0.144 mole) solution of 4,5,6,7-tetrahydro thieno[3,2-c]pyridine in 200 ml of dimethylformamide. The solution is then heated for four hours at 90° C. The reaction mixture is cooled to room temperature, the mineral salts are filtered and the solvent is evaporated. The residue is taken up in water and then extracted with ethylic ether. The ether extracts are washed with water, dried over sodium sulphate and evaporated, to give a yellow oil which is purified by means of its hydrochloride. White crystals. M.p. = 130°-140° C. (ethylacetate, isopropanol). Yield: 45%.

EXAMPLE 2

Methyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]phenylacetate (R_1 = —CH_3; X = H) derivative No. 2.

This compound is prepared by the same procedure described in Example 1 by alkylation of the 4,5,6,7-tetrahydro thieno[3,2-c]pyridine with methyl 2-chlorophenylacetate. Hydrochloride; white crystals softening point = 200° C. (ethanol). Yield: 70%.

EXAMPLE 3

Methyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-α.fluorophenylacetate (R_1 = —CH_3; X = 2-F) derivative No. 3.

This compound is prepared by the same procedure as described in Example 1 by alkylation of 4,5,6,7-tetrahydro thieno[3,2-c]pyridine with methyl 2-chloro-α.fluoro-phenylacetate. Hydrochloride; white crystals; M.p. = 100° C. Yield: 76.5%.

EXAMPLE 4

Ethyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-α.methyl-phenylacetate. (R_1 = —CH_2—CH_3; X = 2-CH_3) derivative No. 4.

This compound is prepared by the procedure described in Example 1 by alkylation of 4,5,6,7-tetrahydro thieno[3,2-c]pyridine with ethyl 2-chloro-α.methyl-phenylacetate. Bisulphate; white crystals: M.p. = 188°-190° C. (isopropanol). Yield: 54%.

EXAMPLE 5

α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-α.chlorophenylacetic acid. (R_1 = H; X = 2-Cl) derivative No. 5.

A mixture of 157.9 g of ethyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-α.chloro-phenylacetate and 100 ml of a 30% sodium hydroxide solution in 600 ml of ethanol is heated under reflux for 2 hours 30 minutes. After evaporation of the ethanol, the mixture is acidified with glacial acetic acid and extracted with methylene chloride. The organic phase is washed with water, dried over magnesium sulphate and then evaporated. After recrystallization in water, the product is isolated in monohydrate form. White crystals; Softening point = 125° C. (water). Yield: 46%.

EXAMPLE 6

α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-phenylacetic acid. (R_1 = H; X = H) derivative No. 6.

This compound is prepared by the procedure described in Example 9 by saponification of ethyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-phenylacetate. The product is purified by means of its sodium salt.

White crystals: M.p. = 210°-215° C. (ethanol, mechanol).

Yield: 74%.

EXAMPLE 7

n-Propyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-α.chloro phenylacetate. (R_1 = —CH_2—CH_2—CH_3; X-2-Cl) derivative No. 7

A stream of hydrochloric gas is bubbled through a 10 g (0.0306 mole) solution of α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-α.chlorophenylacetic acid monohydrate (Example 5) for 12 hours in 100 ml of n-propanol which is refluxed. The mixture is evaporated and the residue is taken up in water, basified with sodium bicarbonate and extracted with ethylic ether. The ether extracts are washed with water, dried over sodium sulphate and evaporated to give a yellow oil which is purified by means of its bisulphate. White crystals. M.p. = 146° C. (crude). Yield: 78%.

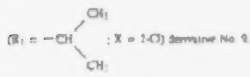
EXAMPLE 8

n-Butyl
α-[4,5,6,7-tetrahydro-thieno[3,2-c] 5-pyridyl]-o.chloro-phenylacetate. (R_1 = —CH_2—CH_2—CH_2—CH_3; X = 2-Cl) derivative No. 8.

This compound is prepared by the procedure described in Example 5 by esterification of α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.-chloro-phenylacetic acid monohydrate (Example 5) with n-butanol. Purified using the bisulphate; white crystals; M p = 155° C. Yield: 79.5%.

EXAMPLE 9

Isopropyl
α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.-chloro-phenylacetate.



(R_1 = —CH(CH$_3$)(CH$_3$); X = 2-Cl) derivative No. 9.

2 ml of thionyl chloride are added dropwise to a 1 g (0.0031 mole) suspension of α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chloro-phenylacetic acid monohydrate (Example 5) in 20 ml of isopropanol cooled to −10° C. The reaction mixture is then refluxed for 6 hours. After evaporation, the residue is taken up in water, basified with sodium bicarbonate and extracted with methylene chloride. The organic phase is washed with water, dried over sodium sulphate and evaporated. A colourless resin is collected and purified using the bisulphate.

White crystals: softening point = 140°-150° C.
Yield: 44%.

EXAMPLE 10

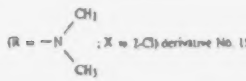
Ethyl
α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chloro-phenylacetate. (R_1 = CH_2—CH_3, X = 2-Cl) derivative No. 10.

4.86 ml (0.051 mole) of ethylchloroformate are added dropwise to a 15 g (0.046 mole) solution of α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.phenylacetic acid monohydrate (Example 5) and 7.12 ml (0.051 mole) of triethylamine in 150 ml of chloroform, cooled to a temperature between −5° and 0° C. The mixture is then allowed to return to room temperature and is stirred for 30 minutes. The reaction mixture is then cooled to a temperature of around 10° C. and 30 ml of ethanol are added dropwise. The reaction mixture is stirred at room temperature for one night and is then washed with water. The organic phase, dried over sodium sulphate, is evaporated to give a colourless oil which is purified by means of its hydrobromide.

White crystals: M p. = 180° C.
Yield: 94%.

EXAMPLE 11

N,N-dimethyl
α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o.chloro-phenylacetamide.



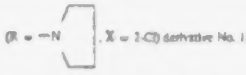
(R = —N(CH$_3$)(CH$_3$); X = 2-Cl) derivative No. 11.

9.72 ml (0.102 mole) of ethyl chloroformate are added dropwise to a 30 g (0.092 mole) solution of α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o.chloro-phenylacetic acid monohydrate and 14.24 ml (0.102 mole) of triethylamine in 300 ml of chloroform, cooled to a temperature between −5° and 0° C. The mixture is then allowed to return to room temperature and is stirred for 30 minutes. The reaction mixture is then cooled to a temperature of around 10° C. and 4.57 ml (0.102 mole) of dimethylamine are added dropwise to 60 ml of chloroform and the mixture is stirred at room temperature for one night. Water is added, the mixture is decanted, and the organic phase is dried over sodium sulphate and evaporated. A colourless resin is collected, which then crystallizes.

White crystals: M.p. = 95°-100° C. (isopropylic ether).
Yield: 49%.

EXAMPLE 12

1-[(2-chloro phenyl)-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl-)acetyl]-pyrrolidine.



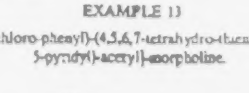
(R = —N(CH$_2$)$_4$; X = 2-Cl) derivative No. 12.

This compound is prepared by the procedure described in Example 11 by condensing α-(4,5,6,7-tetrahydro-thieno(2,3-c)-5-pyridyl]-o.chloro-phenylacetic acid monohydrate with pyrrolidine.

White crystals: M p. = 130° C. (isopropylic ether).
Yield: 61.5%.

EXAMPLE 13

1-[(2-chloro-phenyl)-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-acetyl]-morpholine.



(R = —N(CH$_2$)$_2$O(CH$_2$)$_2$; X = 2-Cl) derivative No. 13.

2.67 g (0.031 mole) of morpholine are added to a 10 g (0.031 mole) solution of α-[4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl]-o.chloro-phenylacetic acid monohydrate and 13.3 g (0.064 mole) of dicyclohexyl carbodiamide in 100 ml of 1,2-dichloro ethane and the mixture is stirred at room temperature for one night. The mixture is evaporated then taken up with 2N hydrochloric acid and ethylic ether. After filtering the dicyclohexylurea formed, the filtrate is decanted and the aqueous phase is basified with 2N sodium hydroxide then extracted with methylene chloride. The organic

phase is washed with water, dried over sodium sulphate and evaporated to give a yellow resin which is purified by means of its hydrochloride hemihydrate.

White crystals: M.p. = 215°-255° C. (isopropanol).

Yield: 71%

EXAMPLE 14

1-[(2-chloro phenyl)-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-acetyl]-piperidine.

(R = —N ; X = 2-Cl) derivative No. 14

This compound is prepared by the procedure described in Example 13 by condensing α-[4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl]-o.chloro-phenylacetic acid monohydrate with piperidine.

White crystals: M.p. = 139° C. (isopropanol).

Yield: 51.5%.

The following compounds were prepared by the process described in Example 11:

[α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl]-o.chlorophenylacetamide. (R = —NH_2; X = 2-Cl). derivative No. 15.

White crystals; M.p. = 126°-128° C. (isopropylic ether-isopropanol).

Yield: 46%.

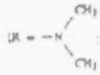
4-benzyl-1-[(2-chloro-phenyl)-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)acetyl] piperazine

(R = —N —CH_2— ; X = 2-Cl) derivative No. 16

oxalate: white crystals. M.p. = 178° C. (ethanol).

Yield: 82.5%.

N,N-dimethyl[α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o.fluoro-phenylacetamide.



(R = —N ; X = 2-F) derivative No. 17

Slightly yellowish powder; M.p. = 125° C. (isopropylic ether-isopropanol).

Yield: 61%.

N-methyl-[α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o chloro-phenylacetamide. (R = NH—CH_3 X = 2-Cl)—derivative No. 18.

White crystals: M.p. = 137° C. (isopropanol).

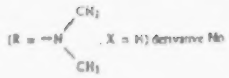
Yield: 85.5%.

N-benzyl[α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o.chloro-phenylacetamide. (R = —NH—(CH_2)—CH_3; X = 2-Cl)—derivative No. 19

White crystals: M.p. = 102° C. (isopropylic ether).

Yield: 55%

N,N-dimethyl[α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)phenylacetamide.

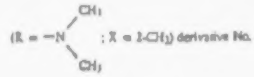


(R = —N ; X = H) derivative No. 20

White crystals: M.p. = 138° C. (isopropylic ether).

Yield: 39%.

N,N-dimethyl[α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o methyl-phenylacetamide.



(R = —N ; X = 2-CH_3) derivative No. 21.

White crystals: M.p. = 119° C. (hexane).

Yield: 15%

The results of pharmacological and toxicological tests reported below demonstrate the properties of the derivatives of the invention both in terms of toxicity and tolerance, and the activity levels of the derivatives, especially their inhibiting action on blood-platelet aggregation and anti-thrombotic activity.

Thus, the invention also relates to a therapeutic composition having in particular an inhibiting action on blood-platelet aggregation and anti-thrombotic action, wherein the active ingredient is a derivative of the formula (I) or an addition salt thereof with a pharmaceutically acceptable mineral or organic acid if Y represents the groups OR or

and with a mineral base if Y is OH.

TOXICOLOGICAL INVESTIGATION

The compounds of the invention exhibit an excellent tolerance and a low toxicity.

In addition, the tests carried out on acute, chronic, sub-chronic and delayed toxicity in various animal species showed no local or general reaction, no disturbances or abnormalities in the biochemical, macroscopic and microscopic examinations performed during this experiment.

PHARMACOLOGICAL INVESTIGATION

(1) Inhibiting action on blood-platelet aggregation

This experiment was performed on rats. The compound to be tested was administered orally in the form of a suspension in gum arabic to the rats over a 3-day period 48 hours, 24 hours and 2 hours before taking a 4 ml blood sample from the animals using Renaud's method from their jugular veins under anaesthetic. The citrated blood was used in the aggregation determinations.

(a) Determination of A.D.P.-induced blood-platelet aggregation.

2 ml of citrated blood were poured quickly into a small beaker placed in a magnetic stirrer, fitted with a magnetic bar. After a few seconds of agitation, 0.4 ml of a solution containing 0.66 mg of adenosine-diphosphate (A.D.P.) per ml was placed in the beaker. After 30 sec-

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onds of stirring, two 0.5 ml samples of blood were taken:

the first was mixed with 0.5 ml of an EDTA-formol solution

the second was mixed with 0.5 ml of a solution of EDTA only

The purpose of adding EDTA formol is to stabilize the blood and thus to fix the aggregation whereas EDTA alone causes disaggregation of all the platelet clusters.

After leaving the mixtures at rest for 10 minutes and centrifuging the two mixtures at low speed for 5 minutes in order to separate the red corpuscles, the supernatant platelet-rich plasma (PRP) was removed, diluted and a platelet count was made.

The intensity of the aggregation was determined by the following ratio:

$$\frac{\text{number of platelets in EDTA-formol}}{\text{number of platelets in EDTA}} \times 100 = \text{percentage of non-aggregated platelets.}$$

The blood-platelet aggregation inhibiting action of the product tested improves as the ratio approaches 100.

The results showing the mean percentage of non-aggregated platelets in groups of 5 rats (treated and reference groups) are shown in Table I

TABLE I

	ADP TEST		
PRODUCT	DOSAGE mg/kg	ROUTE*	RESULT
Reference group	3 x 25	P.O.	16 ± 4
Derivative No. 1		—	94 ± 3
Reference group	3 x 5	—	20 ± 11
Derivative No. 1		—	82 ± 11
Reference group	3 x 2.5	—	23 ± 15
Derivative No. 1		—	36 ± 17
Reference group		—	8 ± 0
Derivative No. 10	3 x 25	—	66 ± 2
Derivative No. 10	3 x 12.5	—	49 ± 11
Reference group	3 x 10	—	8 ± 1
Derivative No. 10		—	24 ± 5
Reference group	3 x 60	—	11 ± 0
Derivative No. 9		—	65 ± 7
Reference group	3 x 100	—	83 ± 3
Derivative No. 4		—	89 ± 1
Reference group	3 x 100	—	3 ± 1
Derivative No. 4		—	89 ± 4
Reference group	3 x 100	—	4 ± 0
Derivative No. 2		—	71 ± 12
Reference group	3 x 100	—	4 ± 0
Derivative No. 12			27 ± 5
Reference group		—	3 ± 0
Derivative No. 17	3 x 100	—	91 ± 3
Derivative No. 20	3 x 100	—	4 ± 1
Reference group	3 x 100	—	18 ± 1
Derivative No. 6		—	22 ± 4

*Route of administration

(b) Determination of collagen induced blood platelet aggregation

0.10 ml of a solution containing 1.0 ug of collagen per ml was added to 1.5 ml of citrated blood. With the mixture being agitated, the platelets were counted continually.

The reduction of the number of free platelets over time was continuously measured and a curve was plotted showing the actual aggregation rate.

The results, representing mean values determined on each group of 5 rats (treated and reference groups), are shown in Table II

TABLE II

	COLLAGEN TEST		
PRODUCT	DOSAGE mg/kg	ROUTE*	RESULT
Reference group	3 x 25	P.O.	3.12 ± 0.41
Derivative No. 1		—	0.14 ± 0.03
Reference group	3 x 5	—	3.17 ± 0.84
Derivative No. 1		—	0.19 ± 0.09
Reference group	3 x 2.5	—	3.60 ± 1.02
Derivative No. 1		—	0.40 ± 0.20
Reference group		—	3.92 ± 0.63
Derivative No. 10	3 x 25	—	0.16 ± 0.07
Derivative No. 10	3 x 12.5	—	0.54 ± 0.12
Reference group		—	3.00 ± 0.35
Derivative No. 9	3 x 100	—	0.46 ± 0.18
Derivative No. 4	3 x 100	—	0.88 ± 0.18
Reference group	3 x 60	—	3.23 ± 0.33
Derivative No. 9		—	0.11 ± 0.01
Reference group	3 x 100	—	3.41 ± 0.55
Derivative No. 4		—	0.17 ± 0.03
Reference group	3 x 100	—	4.73 ± 0.55
Derivative No. 4		—	0.30 ± 0.02
Reference group	3 x 100	—	3.00 ± 1.06
Derivative No. 2		—	0.31 ± 0.18
Reference group	3 x 100	—	3.25 ± 0.12
Derivative No. 12		—	0.83 ± 0.02
Reference group	3 x 100	—	2.77 ± 0.12
Derivative No. 14		—	1.89 ± 0.13
Reference group	3 x 100	—	3.99 ± 0.40
Derivative No. 19		—	2.32 ± 0.68
Reference group	3 x 100	—	3.01 ± 0.79
Derivative No. 21		—	1.04 ± 0.12
Reference group	3 x 100	—	11.35 ± 1.01
Derivative No. 18		—	10.82 ± 0.8

*Route of administration

(c) Determination of bleeding time

The study of the blood-platelet aggregation inhibiting action also concerned the action of the compound of the invention on bleeding time.

The method used is an adaptation of the method of L. STELLA, M. B. DONATI and G. de GAETANO, Thromb. Res., 1975, 7, 709–716.

The experiment was performed on rats. The compound to be tested was administered to the animals per os in suspension in 10 ml/kg of a 5% aqueous solution of gum arabic 65 hours, 41 hours and 17 hours prior to determination. After anesthetizing the animals with pentobarbital, their tails were cut 5 mm from the ends. The blood was carefully sponged at 15-second intervals taking care not to touch the wound.

Hemostasis was reached when the bleeding stopped for one minute.

The results representing mean bleeding times in seconds, determined in each group of 5 rats (reference and treated groups), are given in Table III. Times longer than 1200 seconds (20 minutes) were not counted.

TABLE III

	BLEEDING TIME		
PRODUCT	DOSAGE mg/kg	ROUTE*	RESULT
Reference group		P.O.	600
Derivative No. 10	3 x 100	—	1 200
Derivative No. 2	3 x 100	—	1 200
Reference group		—	420
Derivative No. 1	3 x 25	—	1 200
Derivative No. 1	3 x 5	—	1 020
Reference group	3 x 12.5	—	420
Derivative No. 1		—	1 200
Reference group	3 x 100	—	780
Derivative No. 1		—	1 200
Reference group	3 x 100	—	660
Derivative No. 18		—	1 200
Reference group	3 x 100	—	900

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TABLE III-continued

PRODUCT	BLEEDING TIME DOSAGE mg/kg	ROUTE*	RESULT
Derivative No. 12		—	1 200

*Route of administration

(2) Anti thrombotic activity

This activity was studied by the silk thread experimental thrombosis method.

The principle of this study is an adaptation of the experimental thrombosis method using cardiopulmonary by-pass described by TERUHIKO UMETSU and KAZUKO SANAI (THROMB. HAEMOST., 39. 1. 1978).

The animals' left jugular veins and right external carotid arteries were uncovered (the rats were anesthetized by intraperitoneal injection of pentoburbital.

The arteriovenous shunt consists of a central catheter and two lateral catheters; a white raw silk thread is inserted in the central part and circulation is restored for 20 minutes. After stopping circulation by clamping, the thread is gently withdrawn and weighed immediately. As the average weight of a wet silk thread was previously determined, the weight of the thrombus is determined by evaluation the difference.

The treatment is applied 48 hours, 24 hours and 2 hours before the start of blood flow in the shunt by oral administration of the compound to be tested in suspension in 10 ml/kg of 5% gum arabic, while only the 5% gum arabic solution is administered to the reference group.

The results showing the weight of the thrombus in mg are given in Table IV.

TABLE IV

PRODUCT	DOSAGE mg/kg	ROUTE*	ANTI THROMBOTIC ACTIVITY WEIGHT OF THROMBOSIS IN mg	PERCENTAGE VARIATION
Reference group	3 × 200	P.O.	38.56 ± 3.42	−22
Derivative No. 8		—	29.99 ± 3.05	
Reference group	3 × 200	—	42.65 ± 3.30	−94
Derivative No. 3		—	2.60 ± 0.24	
Reference group		—	36.24 ± 2.05	
Derivative No. 9	3 × 25	—	0.36 ± 0.33	−82
Derivative No. 5	3 × 12.5	—	15.98 ± 1.81	−36
Reference group	3 × 5	—	40.86 ± 2.03	−22
Derivative No. 1		—	27.7 ± 2.82	
Reference group		—	40.68 ± 1.74	
Derivative No. 2	3 × 200	—	8.43 ± 3.28	−79
Derivative No. 10	3 × 200	—	5.89 ± 0.99	−84
Reference group	3 × 200	—	33.76 ± 1.76	−40
Derivative No. 11		—	24.28 ± 4.92	

*Route of administration

The toxicological and pharmacological investigations reported above demonstrate the low toxicity of the compounds of the invention, as well as their excellent tolerance and their inhibiting properties on blood-platelet aggregation, and their anti-thrombotic activity, which make them very useful in medical therapeutic applications.

The drug of the invention may be orally administered in the form of tablets, coated tablets, capsules, drops, granules or syrup. The drug can also be provided in the form of suppositories for rectal administration or in the form of solution for injection for parenteral administration.

Each unit dose contains advantageously from 0.005 g to 0.250 g of a derivative of the invention, the daily dosage regimen varying within a range from 0.005 g to 1.00 g active ingredient according to the age of the patient and the severity of the disease to be treated.

Non-limiting examples of pharmaceutical formulations of the medicine of this invention are given below.

(1) Tablets

Derivative No. 1 . . . 0.050 g

Excipient: lactose, icing sugar, rice starch, alginic acid, magnesium stearate.

(2) Coated tablets

Derivative No. 10 . . . 0.100 g

Excipient: magnesium stearate, corn starch, gum arabic, shellac, white sugar, glucose, white wax, carnauba wax, paraffin, new cochinealin.

(3) Capsules

Derivative No. 17 . . . 0.100 g

Excipient: magnesium stearate, corn starch, lactose

(4) Solution for injection

Derivative No. 4 . . . 0.075 g

isotonic solvent sufficient to make 3 ml

(5) Suppositories

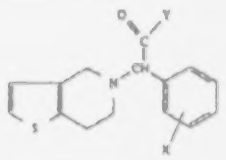
Derivative No. 21 . . . 0.100 g

Semi-synthetic triglycerides sufficient to make 1 suppository.

We claim:

1. A compound of the formula:

(I)



in which Y represents hydroxyl, an OR group wherein R is a straight or branched lower alkyl radical, or

in which R_1 and R_2 are each independent of each other and represent hydrogen or a straight or branched lower alkyl group; or R_1 and R_2 form together with the nitrogen atom to which they are attached a heterocycle selected from the group consisting of pyrrolidino, piperidino, morpholino, piperazino, N-lower alkyl piperazino and N-benzyl piperazino; and X represents hydrogen, a halogen or a lower alkyl radical; and their addition salts with pharmaceutically acceptable mineral or organic acids if Y represents OR groups or

or with mineral bases if Y represents OH, including both enantiomeric forms or their mixture.

2. Methyl α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o.chlorophenyl-acetate.

3. Methyl α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)phenylacetate.

4. Methyl α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o fluoro-phenylacetate.

5. Isopropyl α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o.chloro-phenylacetate.

6. Ethyl α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o.chloro-phenylacetate.

7. N,N-dimethyl-[α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o.chlorophenylacetamide.

8. A therapeutic composition having blood-platelet aggregation inhibiting activities and anti-thrombotic activities containing an effective amount of a compound of claim 1, or an addition salt thereof with a pharmaceutically acceptable mineral or organic acid or with mineral bases, or one of the two enantiomers or their mixture and a pharmaceutically acceptable carrier.

9. The composition as claimed in claim 8, in unit dosage form.

10. The composition as claimed in claim 8 or 9 in unit dosage form, each unit containing from 0.10 g to 1.00 g active ingredient.

11. A method of inhibiting platelet aggregation in mammals which comprises administering an effective amount of a composition comprising a compound of claim 1 and a suitable pharmaceutically acceptable carrier.

12. A method of treating or inhibiting thrombosis in mammals which comprises administering to a mammal in need of treatment an effective amount of a composition comprising a compound of claim 1 and a suitable pharmaceutically acceptable carrier.

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(19) (CA) CANADIAN PATENT (12)
(54) Process for the preparation of thieno[3,2-c]pyridine derivatives and their derivatives

(72) Daniel Aubert
Claude Ferrand
Jean-Pierre Maffrand
France

(73) Assignee: Sanofi, France

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CLAIMS 20 – NO DRAWINGS

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This invention relates to a process for the preparation of thieno[3,2-c]pyridine and to thieno[3,2-c]pyridine derivatives obtained by this process. These thieno[3,2-c]pyridine derivatives have therapeutic applications. The new derivatives, according to the invention, have the following formula:

[Refer to original text for chemical formula.]

(I)

in which Y may represent the OH hydroxyl or the OR group, in which R is a straight or branched lower alkyl radical, or Y represents a group

[Refer to original text for chemical formula.]

in which R_1 and R_2 are each, independently of each other, hydrogen or a straight or branched lower alkyl group; or R_1 and R_2 form together and with the nitrogen atom to which they are attached a heterocycle which may include a second heteroatom such as oxygen or nitrogen, wherein the latter may be substituted by a lower alkyl or benzyl radical which may be substituted; and X represents hydrogen, a halogen or a lower alkyl radical.

These compounds include an asymmetrical carbon which may exist in the form of 2 enantiomers. The invention also concerns each of the enantiomers and their mixture.

The invention also includes the addition salts with pharmaceutically acceptable mineral or organic acids if Y represents the OR groups or

[Refer to original text for chemical formula.]

or with mineral bases if Y represents OH.

The lower alkyl radical refers to a C_1-C_4 saturated hydrocarbon chain.

The invention also relates to a process for the preparation of compounds of formula (I) as defined above, wherein the esters of the invention, in which Y represents an OR group as defined above, are prepared by condensation of 4,5,6,7-tetrahydro-thieno[3,2-c]pyridine, having the following formula:

[Refer to original text for chemical formula.]

(II)

with an α-chlorophenyl acetate having the following formula

[Refer to original text for chemical formula.]

(III)

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in which R and X have the above-defined meanings. Acids of formula (I), in which Y represents the OH hydroxyl, are obtained by saponification.

To prepare the amides, in which Y represents a group

[Refer to original text for chemical formula.]

in which R_1 and R_2 have the above-defined meanings or even some esters of formula (I), the acid of formula (I)(R=OH) is reacted, after activation, if required, either with the amine

[Refer to original text for chemical formula.]

or with the alcohol R-OH.

The α-halogenated esters of formula (III) are prepared using known methods (E.L. ELIEL, M.T. FISK and T. PROSSER, Organic Syntheses, Coll. Vol. IV, J. WILEY and SONS, Inc. New York, 1963, p. 169).

Although it is entirely possible to obtain all the esters of formula (I) by the reaction between the compounds of formulae (II) and (III), it is preferable, for economic reasons, to prepare certain higher esters of formula (I) from the acid of formula (I) and alcohol (R-OH).

The condensation of tetrahydro-thieno-pyridine with the ester of formula (III) is effected in the presence of an alkali metal carbonate, such as potassium carbonate, for example, in an inert solvent, such as dimethylformamide, tetrahydrofuran or 1,2-dimethoxy ethane, at temperatures between 60°C and the boiling point of the solvent.

The saponification of the ester of formula (I), in which R is methyl or ethyl, is effected by an alkali metal hydroxide such as sodium or potassium hydroxide in a hydroalcoholic solvent, at temperatures between room temperature and the boiling point of the solvent.

Activation of the acid of formula (I) may be obtained by treatment with ethyl chloroformate in the presence of a slight excess of triethylamine at temperatures between –5°C and 0°C in an inert solvent such as chloroform, 1,2-dimethoxy ethane or tetrahydrofuran.

A mixed anhydride having the following formula:

[Refer to original text for chemical formula.]

(IV)

is formed, and if treated in situ with a slight excess either of alcohol or amine, at temperatures between 10°C and room temperature, the esters or amides of formula (I) will be formed respectively.

The activation of the acid of formula (I) may also be obtained in different ways: the amides of formula (I) were also thus prepared by condensing the acid (I) with the amine

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[Refer to original text for chemical formula.]

in the presence of dicyclohexycarbodiimide in solution in 1,2-dichloro-ethane.

The esters of formula (I) may also be obtained in the conventional manner by condensing the corresponding acid and alcohol (ROH) in the presence of hydrochloric gas or thionyl chloride.

The following non-limiting examples are given to illustrate the invention.

EXAMPLE 1

Methyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetate (R=-CH_3; X=2-Cl) derivative No. 1

31.47 g (0.144 mole) of methyl 2-chloro-o.chlorophenylacetate and 19.82 g (0.144 mole) of potassium carbonate are added to a 20-g (0.144 mole) solution of 4,5,6,7-tetrahydro-thieno[3,2-c]pyridine in 200 ml of dimethylformamide. The solution is then heated for four hours at 90°C. The reaction mixture is cooled to room temperature, the mineral salts are filtered and the solvent is evaporated. The residue is taken up in water and then extracted with ethylic ether. The ether extracts are washed with water, dried over sodium sulphate and evaporated, to give a yellow oil, which is purified by means of its hydrochloride. White crystals: M.p.=130°-140°C (ethyl acetate, isopropanol). Yield: 45%.

EXAMPLE 2

Methyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-phenylacetate (R=-CH_3; X=H) derivative No. 2

This compound is prepared by the same procedure described in Example 1, by alkylation of the 4,5,6,7-tetrahydro-thieno[3,2-c]pyridine with methyl 2-chloro-phenylacetate. Hydrochloride; white crystals: M.p.=200°C (ethanol). Yield: 50%.

EXAMPLE 3

Methyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.fluorophenylacetate (R=-CH_3; X=2-F) derivative No. 3

This compound is prepared by the same procedure described in Example 1, by alkylation of the 4,5,6,7-tetrahydro-thieno[3,2-c]pyridine with methyl 2-chloro-o.fluorophenylacetate. Hydrochloride; white crystals: Softening point=100°C. Yield: 76.5%.

EXAMPLE 4

Ethyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.methylphenylacetate. (R=-CH_2-CH_3; X=2-CH_3) derivative No. 4

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This compound is prepared by the same procedure described in Example 1, by alkylation of the 4,5,6,7-tetrahydro-thieno[3,2-c]pyridine with ethyl 2-chloro-o.methylphenylacetate. Bisulphate: white crystals: M.p.=188°-190°C (iscpropanol). Yield: 54%.

EXAMPLE 5

α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetic acid. (Y=OH; X=2-Cl) derivative No. 5

A mixture of 157.9 g of ethyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetate and 100 ml of a 30% sodium hydroxide solution in 600 ml of ethanol is heated under reflux for 2 hours 30 minutes. After evaporation of the ethanol, the mixture is acidified with glacial acetic acid and extracted with methylene chloride. The organic phase is washed with water, dried over sodium sulphate and then evaporated. After recrystallization in water, the product is isolated in monohydrate form. White crystals: Softening point = 125°C (water). Yield: 46%.

EXAMPLE 6

α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-phenylacetic acid. (Y=OH; X=H) derivative No. 6

This compound is prepared by the procedure described in Example 9 by saponification of ethyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-phenylacetate. The product is purified by means of its sodium salt. White crystals: M.p. 210°-215°C (ethanol, methanol). Yield: 74%.

EXAMPLE 7

n-Propyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetate (R=-CH_2-CH_2-CH_3; X=2-Cl) derivative No. 7

A stream of hydrochloric gas is bubbled through a 10-g (0.0306 mole) solution of α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetic acid monohydrate (Example 5) for 12 hours in 100 ml of n-propanol, which is refluxed. The mixture is evaporated and the residue is taken up in water, basified with sodium bicarbonate and extracted with ethylic ether. The ether extracts are washed with water, dried over sodium sulphate and evaporated to give a yellow oil, which is purified by means of its bisulphate. White crystals: M.p.=146°C (crude). Yield: 78%.

EXAMPLE 8

n-Butyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetate (R=-CH_2-CH_2-CH_2-CH_3; X=2-Cl) derivative No. 8

This compound is prepared by the procedure described in Example 5 by esterification of α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetic acid monohydrate (Example 5) with n-butanol. Purified using the bisulphate; white crystals: M.p =155°C. Yield: 79.5%.

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EXAMPLE 9

Isopropyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetate (R=-CH[refer to original text for formula]; X=2-Cl) derivative No. 9

2 ml of thionyl chloride are added dropwise to a 1-g (0.0031 mole) suspension of α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetic acid monohydrate (Example 5) in 20 ml of isopropanol cooled to –10°C. The reaction mixture is then refluxed for 6 hours. After evaporation, the residue is taken up in water, basified with sodium bicarbonate and extracted with methylene chloride. The organic phase is washed with water, dried over sodium sulphate and evaporated. A colourless resin is collected and purified using its bisulphate.
White crystals: Softening point=140°-150°C. Yield: 44%.

EXAMPLE 10

Ethyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetate. (R=-CH_2-CH_3; X=2-Cl) derivative No. 10

4.86 ml (0.051 mole) of ethylchloroformate are added dropwise to a 15-g (0.046 mole) solution of α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetic acid monohydrate (Example 5) and 7.12 ml (0.051 mole) of triethylamine in 150 ml of chloroform, cooled to a temperature between –5°C and 0°C. The mixture is then allowed to return to room temperature and is stirred for 30 minutes. The reaction mixture is then cooled to a temperature of around 10°C and 30 ml of ethanol are added dropwise. The reaction mixture is stirred at room temperature for one night and is then washed with water. The organic phase, dried over sodium sulphate, is evaporated to give a colourless oil, which is purified by means of its hydrobromide.
White crystals: M.p.=180°C. Yield: 94%.

EXAMPLE 11

N,N-Dimethyl [α-(4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl)]-o.chlorophenylacetamide

(Y=-N[refer to original text for formula]; X=2-Cl) derivative No. 11.

9.72 ml (0.102 mole) of ethyl chloroformate are added dropwise to a 30-g (0.092 mole) solution of α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetic acid monohydrate and 14.24 ml (0.102 mole) of triethylamine in 300 ml of chloroform, cooled to a temperature between –5° and 0°C. The mixture is then allowed to return to room temperature and the reaction mixture is stirred for 30 minutes. The latter is then cooled to a temperature of around 10°C and 4.57 ml (0.102 mole) of dimethylamine are added dropwise to 60 ml of chloroform and the mixture is stirred at room temperature for one night. Water is added, the mixture is decanted, and the organic phase is dried over sodium sulphate and evaporated. A colourless resin is collected, which then crystallizes.
White crystals: M.p.=95°-100°C (isopropylic ether). Yield: 49%.

EXAMPLE 12

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1-[(2-chlorophenyl)-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl-)acetyl]pyrrolidine

(Y=-N[refer to original text for symbol]; X=2-Cl) derivative No. 12.

This compound is prepared by the procedure described in Example 11 by condensing α-[4,5,6,7-tetrahydro-thieno[2,3-c]-5-pyridyl]-o.chlorophenylacetic acid monohydrate with pyrrolidine.
White crystals: M.p.=130°C (isopropylic ether). Yield: 61.5%.

EXAMPLE 13

1-[(2-chlorophenyl)-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl-)acetyl]morpholine

(Y=-N[refer to original text for symbol]; X=2-Cl) derivative No. 13.

2.67 g (0.031 mole) of morpholine are added to a 10-g (0.031 mole) solution of α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetic acid monohydrate and 13.3 g (0.064 mole) of dicyclohexylcarbodiimide in 100 ml of 1,2-dichloro ethane and the mixture is stirred at room temperature for one night. The mixture is evaporated, then taken up with 2N hydrochloric acid and ethylic ether. After filtering the dicyclohexylurea formed, the filtrate is decanted and the aqueous phase is basified with 2N sodium hydroxide, then extracted with methylene chloride. The organic phase is washed with water, dried over sodium sulphate and evaporated to give a yellow resin, which is purified by means of its hydrochloride hemihydrate.
White crystals: M.p.=215°-225°C (isopropanol). Yield: 71%.

EXAMPLE 14

1-[(2-chlorophenyl)-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl-)acetyl]piperidine

(Y=-N[refer to original text for symbol]; X=2-Cl) derivative No. 14.

This compound is prepared by the procedure described in Example 13 by condensing α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetic acid monohydrate with piperidine.
White crystals: M.p.=139°C (isopropanol). Yield: 51.5%.

The following compounds were prepared by the process described in example 11:

- [α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)]-o.chlorophenylacetamide. (Y=-NH_2; X=2-Cl) derivative No. 15.
White crystals: M.p.=126°-128°C (isopropylic ether-isopropanol).
Yield: 46%.

- 4-benzyl-1-[(2-chloro-phenyl)-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)acetyl]piperazine (Y=-N[refer to original text for formula]; X=2-Cl) derivative No. 16.
Oxalate: white crystals. M.p =178°C (ethanol).
Yield: 82.5%

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- N,N-dimethyl[α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)]-o.fluorophenylacetamide (Y=-N[refer to original text for formula]; X=2-F) derivative No. 17.
Slightly yellowish powder; M.p.=125°C (isopropylic ether-isopropanol). Yield: 41%.

- N-methyl[α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)]-o.chlorophenylacetamide. (Y=NH-CH_3; X=2-Cl) derivative No. 18.
White crystals: M.p.=137°C (isopropanol).
Yield: 85.5%.

- N-butyl[α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)]-o.chlorophenylacetamide. (Y=-NH-$(CH_2)_3$-CH_3; X=2-Cl) derivative No. 19.
White crystals: M.p.=101°C (isopropylic ether).
Yield: 65%.

- N,N-dimethyl[α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)]phenylacetamide. (Y=-N[refer to original text for formula]; X=H) derivative No. 20.
White crystals: M.p.=138°C (isopropylic ether).
Yield: 39%

- N,N-dimethyl[α-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)]-o.methylphenylacetamide. (Y=-N[refer to original text for formula]; X=2-CH_3) derivative No. 21.
White crystals: M.p.=119°C (hexane).
Yield: 15%

The pharmacological and toxicological results reported below demonstrate the properties of the derivatives of the invention both in terms of toxicity and tolerance, and the activity levels of the derivatives, especially their inhibiting action on blood-platelet aggregation and anti-thrombotic activity.

Thus, the purpose of the invention is to obtain a drug having, in particular, an inhibiting action on blood-platelet aggregation and anti-thrombotic action, wherein the active ingredient is a derivative of formula (I) or an addition salt thereof with a pharmaceutically acceptable mineral or organic acid if Y represents the OR groups or $-NR_1R_2$, and with a mineral base if Y is OH.

TOXICOLOGICAL INVESTIGATION

The compounds of the invention exhibit an excellent tolerance and a low toxicity.

In addition, the tests carried out on acute, chronic, sub-chronic and delayed toxicity in various animal species showed no local or general reaction, no disturbances or abnormalities in the biochemical, macroscopic and microscopic examinations performed during this experiment.

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PHARMACOLOGICAL INVESTIGATION

1) Inhibiting action on blood-platelet aggregation

This experiment was performed on rats. The compound to be tested was administered orally in the form of a suspension in gum Arabic to the rats over a 3-day period, 48 hours, 24 hours and 2 hours before taking a 4-ml blood sample from the animals using Renaud's method from their jugular veins under anesthetic. The citrated blood was used in the aggregation determinations.

a) Determination of A.D.P.-induced blood-platelet aggregation

2 ml of citrated blood were poured quickly into a small beaker placed in a magnetic stirrer, fitted with a magnetic bar. After a few seconds of stirring, 0.4 ml of a solution containing 0.66 ug of adenosine-diphosphate (ADP) per ml was placed in the beaker. After 90 seconds of stirring, two 0.5-ml samples of blood were taken:

- the first was mixed with 0.5 ml of an EDTA-formol solution,
- the second was mixed with 0.5 ml of an EDTA only solution.

The purpose of adding EDTA formol is to stabilize the blood and thus to fix the aggregation, whereas EDTA alone causes disaggregation of all the platelet clusters.

After leaving the mixtures to rest for 10 minutes and centrifuging the two mixtures at low speed for 5 minutes in order to separate the red corpuscles, the supernatant platelet-rich plasma (PRP) was removed, diluted and a platelet count was made.

The intensity of the aggregation was determined by the following ratio:

$$\frac{\text{number of platelets in EDTA-formol}}{\text{number of platelets in EDTA}} \times 100 = \text{percentage of non-aggregated platelets.}$$

The blood-platelet aggregation inhibiting action of the product tested improves as the ratio approaches 100.

The results showing the mean percentage of non-aggregated platelets in groups of 5 rats (treated and reference groups) are shown in Table I.

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TABLE I: ADP TEST

PRODUCT	DOSAGE mg/kg	ROUTE	RESULT
Reference group		P.O.	16±4
Derivative No. 1	3 x 25	-	94±3
Reference group		-	20±11
Derivative No. 1	3 x 5	-	82±11
Reference group		-	23±15
Derivative No. 1	3 x 2.5	-	56±17
Reference group		-	8±0
Derivative No. 10	3 x 25	-	66±2
Derivative No. 10	3 x 12.5	-	49±11
Reference group		-	8±1
Derivative No. 10	3 x 10	-	24±5
Reference group		-	11±0
Derivative No. 9	3 x 60	-	65±7
Reference group		-	13±3
Derivative No. 4	3 x 100	-	89±1
Reference group		-	3±1
Derivative No. 4	3 x 100	-	89±4
Reference group		-	4±0
Derivative No. 2	3 x 100	-	72±12
Reference group		-	4±0
Derivative No. 12	3 x 100	-	27±5
Reference group		-	2±0
Derivative No. 17	3 x 100	-	11±3
Derivative No. 20	3 x 100	-	4±1
Reference group		-	18±1
Derivative No. 6	3 x 100	-	22±4

b) Determination of collagen-induced blood-platelet aggregation

0.10 ml of a solution containing 10 µg of collagen per ml was added to 1.5 ml of citrated blood. With the mixture being stirred, the platelets were counted continually.

The reduction of the number of free platelets over time was continuously measured and a curve was plotted showing the initial aggregation rate.

The results, representing mean values determined in each group of 5 rats (treated and reference groups), are shown in Table II.

TABLE II: COLLAGEN TEST

PRODUCT	DOSAGE mg/kg	ROUTE	RESULT
Reference group		P.O.	3.12±0.47
Derivative No. 1	3 x 25	-	0.14±0.03
Reference group		-	2.17±0.64
Derivative No. 1	3 x 5	-	0.19±0.04
Reference group		-	5.00±1.02
Derivative No. 1	3 x 2.5	-	0.60±0.20
Reference group		-	3.92±0.63
Derivative No. 10	3 x 25	-	0.16±0.07
Derivative No. 10	3 x 12.5	-	0.54±0.12
Reference group		-	2.00±0.35
Derivative No. 7	3 x 100	-	0.66±0.18
Derivative No. 8	3 x 100		0.86±0.18
Reference group		-	2.25±0.32
Derivative No. 9	3 x 60	-	0.11±0.01
Reference group		-	3.41±0.55
Derivative No. 4	3 x 100	-	0.12±0.02
Reference group		-	4.73±0.55
Derivative No. 4	3 x 100	-	0.30±0.02
Reference group		-	5.00±1.06
Derivative No. 2	3 x 100	-	0.51±0.18
Reference group		-	2.25±0.32
Derivative No. 11	3 x 100	-	0.83±0.02
Reference group		-	2.77±0.32
Derivative No. 14	3 x 100	-	1.89±0.13
Reference group			3.99±0.40
Derivative No. 17	3 x 100	-	2.22±0.10
Reference group		-	5.01±0.79
Derivative No. 21	3 x 100	-	3.04±0.22
Reference group			11.35±1.01
Derivative No. 18	3 x 100		10.82±0.81

c) Determination of bleeding time

The study of the blood-platelet aggregation inhibiting action also concerned the action of the compound of the invention on bleeding time.

The method used is an adaptation of the method of L. STELLA, M.B. DONATI and G. de GAETANO, Thromb. Res., 1975, 7, 709-716.

The experiment was performed on rats. The compound to be tested was administered to the animals per os in suspension in 10 ml/kg of a 5% aqueous solution of gum Arabic, 65 hours, 41 hours and 17 hours prior to determination. After anesthetizing the animals with pentobarbital, their tails were cut 5 mm from the ends. The blood was carefully sponged at 15-second intervals, taking care not to touch the wound.

Hemostasis was reached when the bleeding stopped for one minute.

The results representing mean bleeding times in seconds, determined in each group of 5 rats (reference and treated groups), are given in Table III. Times longer than 1,200 seconds (20 minutes) were not counted.

TABLE III: BLEEDING TIME

PRODUCT	DOSAGE mg/kg	ROUTE	RESULT
Reference group		P.O.	600
Derivative No. 10	3 x 200	-	>1,200
Derivative No. 2	3 x 200	-	>1,200
Reference group		-	420
Derivative No. 1	3 x 25	-	>1,200
Derivative No. 1	3 x 5	-	1,080
Reference group		-	435
Derivative No. 1	3 x 12.5	-	>1,200
Reference group		-	780
Derivative No. 3	3 x 200	-	>1,200
Reference group		-	600
Derivative No. 18	3 x 200		>1,200
Reference group		-	600
Derivative No. 12	3 x 200	-	>1,200

2) Anti-thrombotic Activity

This activity was studied by the silk thread experimental thrombosis method.

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The principle of this study is an adaptation of the experimental thrombosis method using cardiopulmonary by-pass described by TERUHIKO UMETSU and KAZUKO SANAI (THROMB. HAEMOST., 39, 1, 1978).

Once anesthetized by an intraperitoneal injection of pentobarbital, the rats' left jugular veins and right external carotid arteries were uncovered.

The arteriovenous shunt consists of a central catheter and two lateral catheters; a white raw silk thread is inserted in the central part and circulation is restored for 20 minutes. After stopping circulation by clamping, the thread is gently withdrawn and weighed immediately. As the average weight of a wet silk thread was previously determined, the weight of the thrombus is determined by evaluating the difference.

The treatment is applied 48 hours, 24 hours and 2 hours before the start of blood flow in the shunt by oral administration of the compound to be tested in suspension in 10 ml/kg of 5% gum Arabic, while only the 5% gum Arabic solution is administered to the reference group.

The results showing the weight of the thrombus in mg are given in Table IV.

TABLE IV: ANTI-THROMBOTIC ACTIVITY

PRODUCT	DOSAGE mg/kg	ROUTE	WEIGHT OF THROMBOSIS IN mg	PERCENTAGE VARIATION
Reference group		P.O.	38.56±2.42	
Derivative No. 8	3 x 200	-	29.99±3.05	-22
Reference group		-	42.65±3.30	
Derivative No. 3	3 x 200	-	2.60±0.24	-94
Reference group		-	36.24±2.05	
Derivative No. 1	3 x 25	-	6.56±0.51	-82
Derivative No. 1	3 x 12.5	-	15.98±1.81	-56
Reference group		-	40.86±2.02	
Derivative No. 1	3 x 5	-	27.7±2.82	-32
Reference group		-	40.68±1.74	
Derivative No. 2	3 x 200	-	8.42±3.28	-79
Derivative No. 10	3 x 200	-	5.89±0.99	-86
Reference group		-	35.76±1.76	
Derivative No. 11	3 x 200	-	21.38±2.92	-40

The toxicological and pharmacological investigations reported above demonstrate the low toxicity of the compounds of the invention, as well as their excellent tolerance and their inhibiting properties on blood-platelet aggregation, and their anti-thrombotic activity, which make them very useful in human and veterinary therapeutic applications.

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The drug of the invention may be orally administrated in the form of tablets, coated tablets, capsules, drops, granules or syrup. The drug can also be provided in the form of suppositories for rectal administration or in the form of solution for injection for parenteral administration.

Each unit dose contains advantageously from 0.005 g to 0.250 g of a derivative of the invention, the daily dosage regimen varying within a range from 0.005 g to 1.00 g of active ingredient according to the age of the patient and the severity of the disease to be treated.

Non-limiting examples of pharmaceutical formulations of the drug of this invention are given below.

1. Tablets
Derivative No. 1 0.050 g
Excipient: lactose, icing sugar, rice starch, alginic acid, magnesium stearate.

2. Coated Tablets
Derivative No. 10 0.100 g
Excipient: magnesium stearate, corn starch, gum Arabic, shellac, white sugar, glucose, white wax, carnauba wax, paraffin, new cochinealin.

3. Capsules
Derivative No. 17 0.100 g
Excipient: magnesium stearate, corn starch, lactose.

4. Solution for Injection
Derivative No. 4 0.075 g
Isotonic solvent sufficient to make 3 ml

5. Suppositories
Derivative No. 21 0.100 g
Semi-synthetic triglycerides sufficient to make 1 suppository.

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The findings of the invention, for which an exclusive property and privilege right is claimed, are defined as follows:

1. A process for the preparation of derivatives of general formula (I):

[Refer to original text for chemical formula.]

(I)

in which Y may represent either a hydroxyl radical or an -OR group radical, in which R is a straight or branched lower alkyl radical, or Y represents a radical of the formula:

[Refer to original text for chemical formula.]

in which either R_1 and R_2, identical or different, represent a hydrogen atom or a straight or branched lower alkyl group; or R_1 and R_2 form together and with the nitrogen atom to which they are attached a heterocycle which may or may not include a second heteroatom chosen from the group containing oxygen and nitrogen, wherein the latter may be substituted by a lower alkyl or benzyl radical which may or may not be substituted; and X represents a hydrogen or halogen atom, or a lower alkyl radical; and their the addition salts with pharmaceutically acceptable mineral or organic acids if Y represents a radical of the -OR formula or of the $-NR_1R_2$ formula; R, R_1 and R_2 having the above-defined meanings, or with mineral bases if Y represents a hydroxyl radical; as well as the 2 enantiomers or their mixture of these compounds of formula (I); wherein:

- either, in order to prepare the esters of formula (I), in which Y represents a radical of the –OR formula, in which R has the above-defined meaning, and X has the above-defined meaning, the 4,5,6,7-tetrahydro-thieno[3,2-c]pyridine of formula (II) is condensed:

[Refer to original text for chemical formula.]

(II)

on a α-chlorophenylacetate of formula (III):

[Refer to original text for chemical formula.]

(III)

in which R and X have the above-defined meanings, then the derivative sought is obtained, which is isolated and, if desired, its enantiomers are separated and/or it is salified by mineral or organic acid action;

- or, in order to prepare the acid of formula (I) in which Y represents a hydroxyl radical and X has the above-defined meaning, the ester of formula (I), in which Y represents a radical of the –OR formula in which R and X have the above-defined meanings, is saponified, then the derivative sought is obtained, which is isolated and, if desired, its enantiomers are separated and/or it is salified by mineral or organic acid action:

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- or, in order to prepare the compounds of formula (I), in which X has the above-defined meaning and Y represents a radical of the –OR formula, in which R has the above-defined meaning, or a radical of the $-NR_1R_2$ formula, in which R_1 and R_2 have the above-defined meanings, the acid of formula (I), in which X has the above-defined meaning and Y represents a hydroxyl radical, may or may not be activated, is reacted with either an alcohol of the H-OR formula, in which R has the above-defined meaning, or with an amine of the $H-NR_1R_2$ formula, in which R_1 and R_2 have the above-defined meanings, then the corresponding derivative sought is obtained, which is isolated and, if desired, its enantiomers are separated and/or it is salified by mineral or organic acid action.

2. Process according to claim 1, wherein the condensation reaction between the thieno-pyridine of formula (II) and the ester of formula (III) is effected in the presence of an alkali metal carbonate, in an inert solvent, at temperatures between 60°C and the boiling point of the solvent.

3. Process according to claim 1, wherein the saponification of the ester of formula (I), in which R is methyl or ethyl, is effected by an alkali metal hydroxide in a hydroalcoholic solvent, at temperatures between room temperature and the boiling point of the solvent.

4. Process according to claim 1, wherein the activation of the acid of formula (I) is obtained by ethyl chloroformate in the presence of triethylamine at temperatures between –5°C and 0°C in an inert solvent.

5. Process according to claim 1, wherein the activation of the acid of formula (I) is obtained by ethyl chloroformate in the presence of triethylamine at temperatures between –5°C and 0°C in a solvent chosen in the group containing chloroform, 1,2-dimethoxy ethane or tetrahydrofuran.

6. Process according to claim 1, wherein the activation of the acid of formula (I) for the preparation of amides was effected by dicyclohexycarbodiimide.

7. Process according to claim 1, wherein the activation of the acid of formula (I) for the preparation of esters is effected by a current of hydrochloric gas or thionyl chloride.

8. Process according to claim 1, for the preparation of methyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetate, wherein the 4,5,6,7-tetrahydro thieno[3,2-c]pyridine is condensed over the methyl 2-chloro-o.chlorophenylacetate, and the derivative sought, which is isolated, is obtained.

9. Process according to claim 1, for the preparation of methyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-phenylacetate, wherein the 4,5,6,7-tetrahydro-thieno[3,2-c]pyridine is condensed over the methyl 2-chloro-phenylacetate, and the derivative sought, which is isolated, is obtained.

10 Process according to claim 1, for the preparation of methyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.fluorophenylacetate, wherein the 4,5,6,7-tetrahydro-thieno[3,2-

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c]pyridine is condensed over the methyl 2-chloro-o.fluorophenylacetate, and the derivative sought, which is isolated, is obtained.

11. Process according to claim 1, for the preparation of isopropyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetate, wherein the α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetic acid monohydrate is reacted with isopropanol in the presence of thionyl chloride, and the derivative sought, which is isolated, is obtained.

12. Process according to claim 1, for the preparation of ethyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-chlorophenylacetate, wherein the α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetic acid monohydrate is activated by the action of the ethyl chloroformate in the presence of triethylamine, which is reacted by this acid activated with ethanol, and the derivative sought, which is isolated, is obtained.

13. Process according to claim 1, for the preparation of N,N-dimethyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetamide, wherein the α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetic acid monohydrate is activated by the action of the ethyl chloroformate in the presence of triethylamine, which is reacted by this acid activated with dimethylamine, and the derivative sought, which is isolated, is obtained.

14. Derivatives of general formula (I):

[Refer to original text for chemical formula.]

(I)

in which Y may represent either a hydroxyl radical or an -OR group radical, in which R is a straight or branched lower alkyl radical, or Y represents a radical of the formula:

[Refer to original text for chemical formula.]

in which R_1 and R_2, identical or different, represent a hydrogen atom or a straight or branched lower alkyl group; or R1 and R2 form together and with the nitrogen atom to which they are attached a heterocycle which may or may not include a second heteroatom chosen from the group containing oxygen and nitrogen, wherein the latter may be substituted by a lower alkyl or benzyl radical which may or may not be substituted; and X represents a hydrogen or halogen atom, or a lower alkyl radical; and their addition salts with pharmaceutically acceptable mineral or organic acids if Y represents a radical of the -OR formula or of the $-NR_1R_2$ formula; R, R_1 and R_2 having the above-defined meanings, or with mineral bases if Y represents a hydroxyl radical; as well as the 2 enantiomers or their mixture of these compounds of formula (I); each time they are obtained by the process of claim 1 or its manifest chemical equivalents.

15. Methyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetate, each time it is obtained by the process of claim 8 or its manifest chemical equivalents.

16. Methyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-phenylacetate, each time it is obtained by the process of claim 9 or its manifest chemical equivalents.

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17. Methyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.fluorophenylacetate, each time it is obtained by the process of claim 10 or its manifest chemical equivalents.

18. Isopropyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetate, each time it is obtained by the process of claim 11 or its manifest chemical equivalents.

19. Ethyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-chlorophenylacetate, each time it is obtained by the process of claim 12 or its manifest chemical equivalents.

20. N,N-dimethyl α-[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o.chlorophenylacetamide, each time it is obtained by the process of claim 13 or its manifest chemical equivalents.

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ABSTRACT

The invention concerns a process for the preparation of derivatives of general formula (I):

[Refer to original text for chemical formula.]

(I)

in which Y may represent either an OH hydroxyl or an -OR group, in which R is a straight or branched lower alkyl radical, or Y represents a radical of the formula:

[Refer to original text for chemical formula.]

in which R_1 and R_2 are each, independently of each other, hydrogen or a straight or branched lower alkyl group; or R_1 and R_2 form together and with the nitrogen atom to which they are attached a heterocycle which may or may not include a second heteroatom, such as oxygen or nitrogen, wherein the latter may be substituted by a lower alkyl or benzyl radical which may or may not be substituted; and X represents hydrogen or halogen, or a lower alkyl radical; and their addition salts with pharmaceutically acceptable mineral or organic acids if Y represents the OR groups or

[Refer to original text for chemical formula.]

or with mineral bases if Y represents OH, as well as the 2 enantiomers or their mixture. The invention also concerns the derivatives of formula (I) obtained by the implementation of this process. These derivatives of formula (I) are useful as inhibiting agents of blood-platelet aggregation and anti-thrombotic action.

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AFFIDAVIT

I, Florinda Lages, translator for ALL LANGUAGES LTD, of Toronto, in the Province of Ontario, make oath and say:

1. I understand both the French and the English languages;

2. I have carefully compared the annexed translation from French into English with Canadian Patent Number 1,194,875, issued on October 8, 1985; and

3. The said translation, done by me, is, to the best of my knowledge and ability, a true and correct translation of the said document in every respect.

SWORN before me at the City of)
Toronto, this 6th day of May)
A.D. 2002.)
)
)
A Notary Public in and for the)
Province of Ontario.)

MAURICE PENZO, Notary Public, City of Toronto, limited to the attestation of instruments and the taking of affidavits, for All Languages Ltd.
Expires December 12, 2004

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United States Patent [19]

Badorc et al.

[11] **Patent Number: 4,847,265**

[45] **Date of Patent: Jul. 11, 1989**

[54] **DEXTRO-ROTATORY ENANTIOMER OF METHYL ALPHA-5 (4,5,6,7-TETRAHYDRO (3,2-C) THIENO PYRIDYL) (2-CHLOROPHENYL)-ACETATE AND THE PHARMACEUTICAL COMPOSITIONS CONTAINING IT**

[75] Inventors: **Alain Badarc, Roquettes; Daniel Fréhel, Toulouse**, both of France

[73] Assignee: **Sanofi**, France

[21] Appl. No.: **155,550**

[22] Filed: **Feb. 12, 1988**

[30] **Foreign Application Priority Data**

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Nov. 27, 1987 [FR] France 87 16516

[51] **Int. Cl.**[4] **A61K 31/44; C07D 495/04**
[52] **U.S. Cl.** **514/301; 546/114**
[58] **Field of Search** 546/114; 514/301

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,529,596 7/1985 Aubert et al. 546/115

FOREIGN PATENT DOCUMENTS

0099802 7/1983 European Pat. Off. .

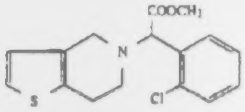
OTHER PUBLICATIONS

Fieser et al., Advanced Org. Chem.-Reinhold Publishing Co., N.Y., (1961), pp. 85-88.

Primary Examiner—Mary C. Lee
Assistant Examiner—Bernard I. Dentz
Attorney, Agent, or Firm—Wegner & Bretschneider

[57] **ABSTRACT**

The present invention relates to the dextro-rotatory enantiomer of Formula



and its pharmaceutically acceptable salts with platelet aggregation inhibiting activity.

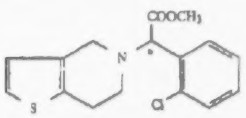
The invention also relates to a process for the preparation of this compound starting from the racemate and the pharmaceutical compositions containing it.

7 Claims, No Drawings

DEXTRO-ROTATORY ENANTIOMER OF METHYL ALPHA-5 (4,5,6,7-TETRAHYDRO (3,2-C) THIENO PYRIDYL) (2-CHLOROPHENYL)-ACETATE AND THE PHARMACEUTICAL COMPOSITIONS CONTAINING IT

The present invention relates to the dextro-rotatory enantiomer of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate, a process for its preparation and pharmaceutical compositions containing it.

The compound of the invention corresponds to the following formula (I):



(I)

in which the C* is an asymmetric carbon atom. In fact, this formula represents both the dextro-rotatory molecule claimed as well as its levo-rotatory enantiomer. The racemic mixture corresponding to this formula was described in the French patent application published under the No. 2 530 247. Hereafter the dextro-rotatory enantiomer claimed according to the invention will be designated by I_d and the levo-rotatory enantiomer by I_l.

It is known that the optical rotatory power of a compound depends on the solvent in which it is measured and on its concentration in this solvent. The optical rotatory power of the dextro-rotatory isomer according to the invention is positive in methanolic solution.

In an unexpected manner only the dextro-rotatory enantiomer I_d exhibits a platelet aggregation inhibiting activity, the levo-rotatory enantiomer I_l being inactive. Moreover, the inactive levo-rotatory enantiomer I_l is the less well tolerated of the two enantiomers.

The invention also relates to the addition salts of the compounds of formula (I_d) with pharmaceutically acceptable mineral or organic acids.

The compound (I_d) is an oil whereas its hydrochloride exists as a white powder. The oily products are usually difficult to purify and it is preferable to use for the preparation of pharmaceutical compositions crystalline products which can usually be purified by recrystallization.

However, it has been observed in the present case that some of the salts of compound (I_d) usually precipitate in an amorphous form and/or that they are hygroscopic, a property which makes them difficult to handle on an industrial scale. Thus, the salts of carboxylic acid and sulfonic acids classically used in pharmacy have been prepared, acids such as acetic, benzoic, fumaric, maleic, citric, tartaric, gentisic, methane-sulfonic, ethanesulfonic, benzenesulfonic and laurylsulfonic acids as well as the salts of dobesilic acid (m.p. = 70° C.) and para-toluenesulfonic acid (m.p. = 51° C.), the purification of which proved to be difficult.

Among the mineral and organic acid salts of the dextro-rotatory isomer of the compound of Formula (I_d) salts have been found which crystallize easily, are not hygroscopic and are sufficiently water-soluble as to make their use as active medicinal principles particularly advantageous.

The present invention thus relates more particular to the hydrogen sulfate, the taurocholate and the hydr bromide of the dextro-rotatory enantiomer of meth alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (chlorophenyl)-acetate.

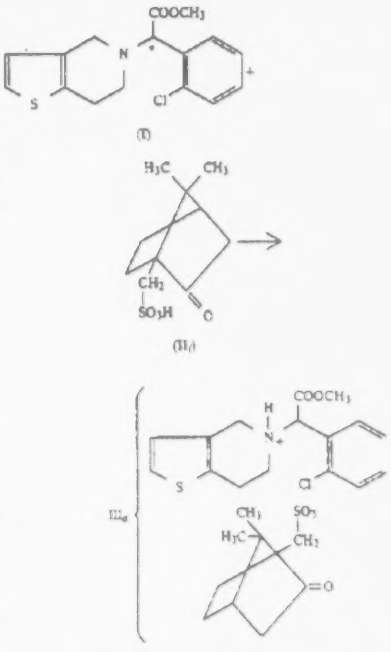
These salts are prepared in a standard manner by t action of the corresponding acid on the base in soluti in a solvent from which they precipitate spontaneous or after addition of a non-solvent of the salt.

The dextro-rotatory isomer of methyl alpha-5(4,5,6, tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-ac tate can be prepared by forming the salt of the racem compound with an optically active acid in a solve repeated recrystallizations of the salt until a product constant optical rotatory power is obtained, follow by the liberation of the dextro-rotatory isomer from salts by a base; if required, a salt is formed between t dextro-rotatory isomer and a pharmaceutically acce able acid.

The optically active acid may advantageously levo-rotatory camphor-10-sulfonic acid.

One and the same solvent may be used for salt form tion and recrystallization: acetone is ideally suited this case.

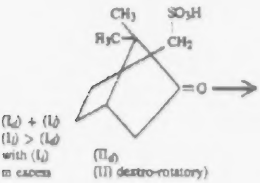
The chiral, levo-rotatory camphor-10-sulfonic acid Formula (II$_l$) is allowed to react in an inert solvent w the racemic mixture of Formula (I) according to t following reaction scheme:

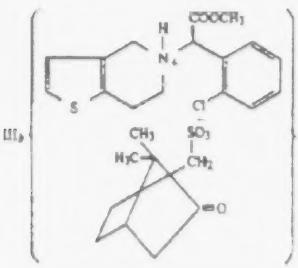


Salt formation may be carried out in solvents such alcohols, ketones, dimethylformamide. The salt prec tates spontaneously or is isolated by salting out or ev oration of the solvent. A mixture of two diastereoi mers of Formula (IIIa) is formed. By repeated recrys lizations from a solvent such as acetone the precipit

is enriched in the salt of the dextro-rotatory isomer of compound (I). After each recrystallization the optical rotatory power $[\alpha]_D^{20}$ of the precipitate is measured at 20° C. in methanol at a concentration varying between 1.5 and 2 g/100 mL When the $[\alpha]_D^{20}$ stops increasing the base of Formula (I_D) is liberated from the salt (IIIa) by the action of a base such as sodium or potassium hydrogen carbonate in aqueous media at temperatures varying between 5° C. and 20° C. Evaporation of the filtrate of the first recrystallization IV after the crystals of the precipitated salt (IIIa) have been filtered off, gives a mixture enriched in the salt of (I_l) enantiomer. The basification of this mixture of diastereoisomeric salts with a weak base such as sodium or potassium hydrogen carbonate in aqueous solution at temperatures varying between 5° C. and 20° C. leads to a mixture of the two enantiomers (I_d) plus (I_l) enriched in the levo-rotatory enantiomer (I_l).

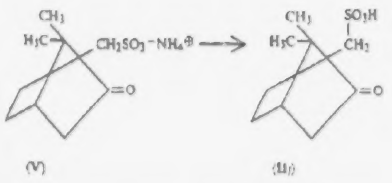
This mixture (I_d)+(I_l) enriched in enantiomer (I_l) is allowed to react with dextro-rotatory camphor-10-sulfonic acid which will be designated as (II_d) in a solvent according to the following reaction scheme:





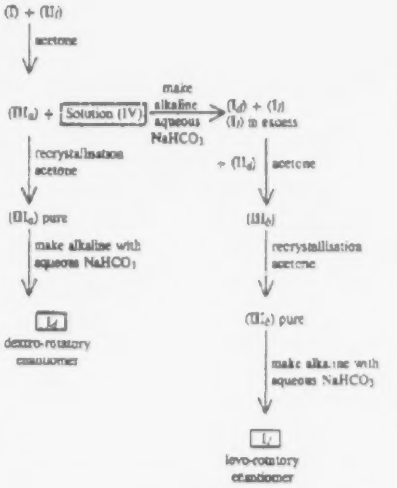
The crystalline mixture of diastereoisomeric salts (IIIb) obtained is recrystallized from acetone until the optical rotatory power $[\alpha]_D^{20}$ of the precipitate remains constant. As previously mentioned the optical rotatory power $[\alpha]_D^{20}$ of the diastereoisomeric salt (IIIb) is determined after each recrystallization.

The liberation of the stereoisomeric (I_l) from its salt is carried out in a standard manner, like that for compound (I_d). Levo-rotatory camphor-10-sulfonic acid (II_l) may be obtained from commercially available ammonium camphor-10-sulfonate of Formula (V) according to the reaction scheme:



An aqueous solution of the ammonium salt (V) is chromatographed on an Amberlite IRN-77 resin. After lyophilization of the eluate camphor-10-sulfonic acid of Formula (II_l) is recovered.

The entire sequence of the process is shown schematically below:



Each of the pure enantiomers (I_d) and (I_l) may be converted into a salt by means of the standard methods. for example, the hydrochlorides are prepared by the addition of a solution of hydrogen chloride gas in diethyl ether to a solution of (I_d) or (I_l) in diethyl ether.

DETERMINATION OF THE ENANTIOMERIC PURITY OF THE DEXTRO-ROTATORY (I_d) AND LEVO-ROTATORY (I_l) ENANTIOMERS

Two methods have been used:

- proton NMR spectroscopy with the addition of a chiral rare earth
- high pressure liquid chromatography using a chiral stationary phase.

(a) Proton NMR spectroscopy with the addition of a chiral rare earth The enantiomeric purity (optical purity) was determined by ^1H 60 MHz NMR spectroscopy in the presence of a rare earth chiral complex according to the method described by G. M. WHITESIDES et al. (J. Am. Chem. Soc. 1974, 96, 1038).

In the racemic product (I), the hydrogen attached to the asymmetric centre in the α position to the ester function appears as a singlet (chemical shift $\delta = 4.87$ ppm in $CDCl_3$ as solvent. The addition of the rare earth complex Eu(tfc)$_3$ [tris 3-(trifluoromethyl hydroxyme-

thylene)-d-camphorato europium (III)] to the probe containing a solution of the racemate (I) in $CDCl_3$ leads to the resolution of the initial singlet into two, well-separated singlets corresponding to the proton of each of the two enantiomers (I_d) and (I_l). When the molar ratio of rare earth complex/compound (I)=0.4, the separation between the two singlets is about 6 Hz.

With each of the two enantiomers prepared, (I_d) and (I_l), the same procedure was used as for the racemate (I). The smaller chemical shift corresponds to the proton of the dextro-rotatory enantiomer (I_d) and the larger chemical shift to the levo-rotatory enantiomer (I_l).

The precision of the method was determined by comparing the ^1H (60 MHz) NMR spectra obtained with and without addition of the rare earth complex for each of the two enantiomers (I_d) and (I_l) in the pure state or as mixtures containing increasing quantities of one of the enantiomers. It was observed that it was possible to detect easily more than 5% by weight of one enantiomer in the presence of the other.

(b) High pressure liquid chromatography using a chiral stationary phase The study was conducted with a liquid chromatograph HP-1084 using a UV detector at 215 nm. The chiral stationary phase was DEAE silica (10 microns) onto which was grafted alpha-1 acid glycoprotein (0.4×100 mm) (ENANTIOPAC R-LKB). The mobile phase consisted of an aqueous phosphate buffer mixture 8 mM (NaH_2PO_4/Na_2HPO_4) containing 0.1M of NaCl, adjusted to pH=7.4, and 15% of isopropanol (v/v). The flow rate was fixed at 0.3 ml/minute and the temperature of the column was maintained at 18°-20° C. Under these conditions, the dextro-rotatory enantiomer (I_d) has a retention time of 45 minutes and the levo-rotatory enantiomer (I_l) has a retention time of 35 minutes.

The precision of the determination of the optical purity of the two enantiomers was estimated by chromatographing each of the two enantiomers (I_d) and (I_l) prepared either alone or as a mixture containing increasing amounts of one of the enantiomers. It was observed that it was easy to detect:

2% (weight/weight) of enantiomer (I_d) in enantiomer (I_l)

4% (weight/weight) of enantiomer (I_l) in enantiomer (I_d).

Under these conditions it may be concluded that the optical purity of the two enantiomers (I_d) and (I_l) obtained according to the examples is at least equal to 96% for the dextro-rotatory enantiomer (I_d) and at least equal to 98% for the levo-rotatory enantiomer (I_l).

The following examples are non-restrictive and are presented to illustrate the present invention.

EXAMPLE I

Salts of dextro-rotary methyl-alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate

(a) levo-rotatory camphor-10-sulfonic acid

A column of Amberlite IRN-77 resin is prepared and tested by passing 1N hydrochloric acid through it and then by washing this column of acidified resin abundantly with water. Levo-rotatory ammonium camphor-10-sulfonate is dissolved in a minimum of water and applied to the column of resin previously prepared. Elution is carried out with water. The eluted fractions containing the acid are lyophilized.

White crystals, m.p.=198° C.; $[\alpha]_D^{20}=-20.53$ (c=2.075 g/100 ml water).

(b) Camphor-10-sulfonic acid salt of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate (SR 25990 B).

32 g (0.0994 mole) of racemic methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate are dissolved in 150 ml of acetone. 9.95 g (0.0397 mole) of levo-rotat camphor-10-sulfonic acid monohydrate are added. The clear solution is left to stand at room temperature. After 48 hours some crystals have formed. The reaction mixture is concentrated to 50 ml and left to stand at room temperature for 24 hours. The crystals obtained are filtered off, washed with acetone and dried (yield: 55% on the basis of the starting racemate).

White crystals, m.p.=165° C., $[\alpha]_D^{20}=+24.67$ (c=1.58 g/100 ml; methanol).

The crystals obtained above are redissolved in the minimum of boiling acetone (50 ml). The crystals obtained after cooling are filtered off, washed with acetone and dried (yield: 88%).

White crystals, m.p.=165° C., $[\alpha]_D^{20}=+24.75$ (c=1.68 g/100 ml; methanol).

(c) Dextro-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate

12 g (0.022 mole) of the pure product obtained in (b) are dissolved in a minimum of water. After cooling to 5° C., the aqueous solution obtained is made alkaline with a saturated aqueous solution of sodium hydrogen carbonate. The alkaline aqueous phase is extracted with dichloromethane. The organic extracts are dried over anhydrous sodium sulfate. On evaporation of the solvent a colorless oil is obtained (quantitative yield). Oil, $[\alpha]_D^{20}=+51.52$ (c=1.61 g/100 ml; methanol).

(d) The hydrochloride of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate (dextro-rotatory) (SR 25990 A).

7 g (0.0228 mole) of dextro-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate are dissolved in 100 ml of diethyl ether. 30 ml of a solution of 1N HCl in diethyl ether are added and the crystals obtained are filtered off. The crystals are washed with diethyl ether and dried (yield: 94%).

White crystals, m.p.=117° C., $[\alpha]_D^{20}=+62.23$ (c=1.82 g/100 ml; methanol).

(e) The hydrogen sulfate of dextro-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate (SR 25990 C).

800 ml of a saturated aqueous solution of sodium bicarbonate are added to a suspension of 200 g of SR 25990 B in 800 ml of dichloromethane. After vigorous shaking, the organic phase is separated, dried over sodium sulfate and the solvent is removed under reduced pressure. The residue is dissolved in 500 ml of ice-cold acetone and 20.7 ml of concentrated sulfuric acid (93.64%, d=1.83) are added drop-wise. The precipitate formed is isolated by filtration and washed with 1,000 ml of acetone, then dried in a vacuum oven at 50° C.

139 grams of analytically pure white crystals are thus obtained with a melting point of 184° C. Empirical formula $C_{16}H_{18}ClNO_2S.H_2SO_4$ $[\alpha]_D^{20}=+55.10$ (c=1.89 g/100 ml; methanol).

(f) The hydrochloride of dextro-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate (SR 25990D).

150 ml of an aqueous solution of sodium bicarbonate are added to a suspension of 20 g of SR 25990 B in 200

ml of dichloromethane. The residue obtained after separation of the organic phase, drying and evaporation of the solvent is dissolved in 150 ml of diethyl or diisopropyl ether, and 4.4 ml of a 48% (wt/v) aqueous solution of hydrobromic acid are added drop-wise. The precipitate formed is isolated. After drying, 14.4 g of crystals are obtained with a melting point of 111° C. (yield 99%).

13.4 g of these crystals are recrystallized from a mixture of isopropyl ether (100 ml) and isopropanol (150 ml) to give 10.2 g of analytically pure hydrobromide: m.p. = 140° C.; empirical formula: $C_{16}H_{16}ClNO_2S.HBr$ $[\alpha]_D^{20} = +59.23$ (c = 2.09 g/100 ml; methanol).

(g) The taurocholate of dextro-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate (SR 25990E).

The sodium salt of taurocholic acid is chromatographed on Amberlite IRN-77 resin by eluting with water. The fractions obtained are lyophilized.

3 g (0.0054 mole) of SR 25990B are treated with a saturated aqueous solution of sodium bicarbonate and the mixture is extracted with dichloromethane. The organic phase is separated, dried over sodium sulfate and evaporated to dryness. The free base obtained is taken up in 30 ml of isopropanol; 2.8 g (0.0054 mole) of taurocholic acid dissolved in 100 ml of isopropanol are added to this solution. The mixture is stirred overnight at room temperature, then evaporated to dryness. The residue solidifed on being triturated with ether. 3.5 g of beige crystals are obtained. m.p. = 120° C. $[\alpha]_D^{20} + = 39.53$ (c = 1.791 g/100 ml of methanol). $C_{16}H_{16}ClNO_2S.C_{26}H_{45}NO_7S$; C, H, N analyses in agreement with theory.

EXAMPLE 2

Salts of levo-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate

(a) Salt of d-camphor-10-sulfonic acid (SR 25989 B)

After separation of SR 25990 B in Example 1-b the solvent is evaporated from the acetone filtrate obtained.

The residue is taken up in water and diethyl ether. The ethereal phase is decanted. The aqueous phase is cooled to 5° C. and made alkaline with a saturated aqueous solution of sodium bicarbonate. The aqueous alkaline phase is extracted with diethyl ether. The ethereal extracts are pooled and dried over anhydrous sodium sulfate.

On evaporation of the solvent an oil is obtained which is purified by filtration through a bed of silica (eluant: diethyl ether). A colourless oil is recovered consisting of a mixture of about 65% of the levo-rotatory enantiomer and 35% of the dextro-rotatory enantiomer, proportions determined by means of ^1H (60 MHz) NMR spectroscopy after the addition of chiral, rare earth complex.

16.66 g (0.0517 mole) of the mixture thus obtained are dissolved in 70 ml of acetone. 7.77 g (0.0310 mole) of dextro-rotatory camphor-10-sulfonic acid monohydrate are added. The homogeneous mixture is left to stand overnight at room temperature. The crystals obtained are filtered off, washed with acetone and dried (yield: 44% based on the mixture).

The crystals obtained are dissolved in a minimum of refluxing acetone (60 ml). The precipitate obtained on cooling to room temperature is filtered off, washed with acetone and dried. White crystals, m.p. = 167° C., $[\alpha]_D^{20} = -24.85$ (c = 1.79 g/100 ml; methanol).

(b) Levo-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate.

11.3 g (0.0204 mole) of the camphor-10-sulfonate obtained in (a) are dissolved in a minimum of water. The aqueous solution obtained is cooled to 5° C. and made alkaline with a saturated aqueous solution of sodium hydrogen carbonate. The alkaline aqueous phase is extracted with dichloromethane. The organic solution is dried and the solvent is evaporated. A colourless oil is isolated (quantitative yield).

Oil $[\alpha]_D^{20} = -50.74$ (c = 1.58 g/100 ml, methanol).

(c) The hydrochloride of levo-rotatory methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate (SR 25989 A).

Prepared according to the method described in Example 1(d). Yield: 94%.

White crystals, m.p. = 117° C., $[\alpha]_D^{20} = -62.56$ (c = 1.80 g/100 ml; methanol).

(d) The hydrogen sulfate of levo-rotatory methyl-alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate (SR 25989 C).

70 g (0.126 mole) of the camphor sulfonate SR 25989 B obtained are treated as described in (a) above with a saturated aqueous solution of sodium bicarbonate in the presence of dichloromethane. The organic phase is separated, dried over sodium sulfate and evaporated to dryness. The residue is taken up in 300 ml of acetone and 7.2 ml (0.126 mole) of concentrated sulfuric acid are added drop-wise. After being stirred the crystals formed are filtered off and washed with acetone. 47.8 g of white crystals are obtained. m.p. = 182° C. $[\alpha]_D^{20} = -51.61$ (c = 2.044 g/100 ml; methanol). The analysis (C,H,N) are in agreement with theory.

PHARMACOLOGICAL STUDY

The platelet aggregation inhibiting activity and the toxicity of these new compounds were compared to those of the racemic mixture described in the French Pat. No. 82.12599 (Publication No. 2 530 247).

A description will now be given of the results of this study which demonstrates another advantage of the invention, namely that the salts of the dextro-rotatory isomer have a better therapeutic index than the salt of the racemic mixture; in fact, the levo-rotatory isomer exhibits almost no platelet aggregation inhibiting activity and its toxicity is markedly higher than that of its dextro-rotatory homologue.

The platelet aggregation inhibiting activities and the antithrombotic activities of the compounds were studied in the rat by standard methods.

The activity on the aggregation of plates induced by ADP or collagen was determined ex-vivo.

The products dissolved in ethanol (200 mg/ml) and diluted in water containing gum arabic (5%-wt/v) were administered by the oral route to groups of five female rats of the CD-COBS strain, weighing 250-300 g, in amounts of 10 ml of suspension per kilogram two hours before blood samples were taken.

The blood samples were taken from animals anesthetized with diethyl ether by puncture of the abdominal aorta and placed over a 3.8% aqueous solution of sodium citrate (1 vol/9 volumes of blood). The platelet-rich plasma was then isolated by centrifugation at 200 g for 10 minutes.

Aggregation is induced by the addition of 2 µl of aggregating solution to 400 µl of platelet-rich plasma.

The aggregating solutions used were: a 500 μM aqueous solution of ADP marketed by Boehringer Mannheim (final concentration 2.5 μM), and a solution of collagen marketed by Sigma (type 1) at a concentration of 0.25 g/100 ml in 3% acetic acid (v/v) (final concentration 12.5 μg/ml).

The aggregation of the platelets was monitored as described in the method by G. V. R. Born in Nature 194, p. 927 (1967) using a Coultronics ® aggregometer at a temperature of 37° C. and agitation of 900 rpm.

For aggregation with ADP, the aggregometer generates a curve representing a platelet aggregation as measured by a change in optical density. The height of this curve is defined as the height of aggregation. The percentage of aggregation is the relation between the aggregation height measured and the height corresponding to 100% aggregation × 100. The percentage of inhibition is determined by the relation:

$$\frac{\text{Control aggregation height} - \text{produced aggregation height}}{\text{Control aggregation height}} \times 100$$

The results obtained for the aggregation with ADP for the hydrochloride of the racemic mixture (PCR 4099), the hydrogen sulfates of the dextro-rotatory (SR 25990 C) and levo-rotatory (SR 25989 C) isomers on the one hand, and for PCR 4099 and the hydrochlorides of the dextro-rotatory (SR 25990 A) and levo-rotatory (SR 25989 A) on the other, are shown in Table I; they demonstrate that the levo-rotatory isomer is inactive and that the dextro-rotatory isomer is at least as active as the racemate.

TABLE I

PRODUCT	DOSE mg/Kg P.O	QUANTITY of base administered		% INHIBITION	p**
			% AGGREGATION		
Controls			42.4 +/- 1.5		
PCR 4099	4.48	3.84	29.8 +/- 2.4	30	0.01
(racemate)	8.97	7.69	17.2 +/- 2.2	59	0.001
	17.9	15.38	11.1 +/- 2.3	74	0.001
SR 25989C	20	15.38	41.0 +/- 1.5	3	n.s
	40	30.76	37.1 +/- 1.7	13	n.s
SR 25990C	1.25	0.96	39.4 +/- 1.3	7	n.s
	2.5	1.92	28.4 +/- 2.3	33	0.01
	5	3.84	14.0 +/- 1.6	67	0.001
	10	7.69	8.5 +/- 1.6	80	0.001
Controls			33.8 +/- 2.3		
SR 25990E	10	3.84	9.6 +/- 3	72	0.001
	20	7.69	4 +/- 1.6	88	0.001
			AGGREGATION HEIGHT		
Controls			103 +/- 5		
PCR 4099	2.5	2.14	86 +/- 5	17	0.05
(racemate)	5	4.28	72 +/- 8	30	0.05
	12.5	10.71	32 +/- 9	69	0.001
SR 25989A	25	22.46	100 +/- 1	3	n.s
SR 25990A	2.5	2.25	67 +/- 7	35	0.01
	5	4.49	26 +/- 5	75	0.001
	12.5	11.23	19 +/- 4	82	0.001
	25	22.46	11 +/- 1	89	0.001

*mean of results +/- standard error of the mean (SEM)
**Student test
***aggregation height in mm mean +/- SEM (n = 5)
n.s. not significant

For the aggregation with collagen, the percentage of inhibition is the difference of the slopes of the curves representing the variation of the optical density as a function of time for the control and the product to be tested divided by the slope of the control multiplied by 100. The results shown in Table II demonstrate again that only the dextro-rotatory isomer is active whereas the salts have comparable activities.

TABLE II

PRODUCT	DOSE mg/Kg P.O	QUANTITY of base administered	SLOPE	% INHIBITION	P**
Controls			4.8 +/- 0.3		
PCR 4099	4.48	3.84	3.6 +/- 0.2	25	0.05
(racemate)	8.97	7.69	2.7 +/- 0.3	44	0.01
	17.9	15.38	1.5 +/- 0.3	69	0.001
SR 25989C	20	15.38	4.3 +/- 0.2	10	n.s
	40	30.76	4.0 +/- 0.2	17	n.s
SR 25990C	1.25	0.96	4.3 +/- 0.3	6	n.s
	2.5	1.92	4.1 +/- 0.2	15	n.s
	5	3.84	2.3 +/- 0.1	52	0.001
	10	7.69	1.7 +/- 0.3	65	0.001
Controls			3.5 +/- 0.1		
SR 25990E	10	3.84	2.1 +/- 0.5	60	0.05
	20	7.69	1.4 +/- 0.4	60	0.01
Controls			3.97 +/- 0.29		
PCR 4099	2.5	2.14	3.13 +/- 0.33	21	n.s
(racemate)	5	4.28	2.94 +/- 0.36	26	0.05
	12.5	10.71	2.19 +/- 0.42	45	0.01
SR 25989A	25	22.46	4.32 +/- 0.29	10	n.s
SR 25990A	2.5	2.25	3.05 +/- 0.19	23	0.05
	5	4.49	1.24 +/- 0.22	69	0.001
	12.5	11.23	0.86 +/- 0.14	78	0.001
	25	22.46	0.74 +/- 0.13	81	0.001

**Student test
n.s. not significant

The antithrombotic activity of these compounds has also been studied in the test of venous thrombosis on a screw thread described by Kumada T. et al. in Thromb. Res 18 p. 189 (1980).

Female rats of the same type as those previously described, in groups of 10 animals, were anesthetized with diethyl ether and their vena cava was isolated after abdominal incision.

A metallic screw thread 21 mm long consisting of a dentist's drill, marketed by Dyna (France) size No. 30, was introduced into the lumen of this vein just below the renal bifurcation descending towards the iliac veins, without damaging the wall; 19 to 20 mm of the length of the screw thread are implanted and the remaining 1 mm protrudes through the closed stomach into the exterior.

The thrombi formed rapidly and five hours later, under pentobarbital anesthesia, the abdomen is reopened and ligatures are placed above and below the screw thread which is withdrawn after longitudinal incision of the vein and the isolated thrombus is weighed.

The results which are presented in Table III show that the levo-rotatory isomer is inactive in this test, in contrast to the dextro-rotatory isomer and the racemate.

TABLE III

PRODUCT	DOSE mg/Kg P.O admin.	QUANTITY of base	WEIGHT of thrombi*	VARIATION %	P**
Controls			3.9 +/- 0.3		
PCR 4099	4.48	3.84	2.17 +/- 0.24	44	0.001
(racemate)	8.97	7.69	1.39 +/- 0.19	64	0.001
	17.9	15.38	1.00 +/- 0.19	74	0.001
SR 25989C	40	30.76	4.17 +/- 0.42	-7	n.s
SR 25990C	1.25	0.96	3.11 +/- 0.32	20	n.s
	2.5	1.92	2.29 +/- 0.22	41	0.01
	5	3.84	1.71 +/- 0.24	56	0.01
	10	7.69	1.26 +/- 0.19	67	0.01
	20	15.38	1.20 +/- 0.13	69	0.01

TABLE III-continued

PRODUCT	DOSE mg/Kg P.O admin.	QUANTITY of base	WEIGHT of thrombi*	VARIATION %	P**
Controls			3.78 +/− 0.36		
SR 25990E	10	3.84	1.48 +/− 0.15	60	0.001
	20	7.69	1.16 +/− 0.15	68	0.001

* = weight of thrombi in mg +/− standard error of the mean
P = U test of Kruskal - Wallis

For the toxicological study, the compounds were administered by the oral route in the form of a suspension in the same volume of water made up to 10% (wt/v) with gum arabic to groups of 10 fasted female rats of the Sprague Dawley strain weighing 120 to 135 grams.

The number of dead animals was determined 14 days after the administration of the compound under study. The lethal doses thus determined, expressed in weight of the salt administered, are presented in Table IV; these results show on the one hand that the toxicity of the racemic mixture is similar to that of the levo-rotatory isomer whereas the dextro-rotatory isomer is markedly less toxic, and, on the other hand, that the toxicity depends on the nature of the acid used to form the salt.

TABLE IV

PRODUCTS	D 10	D 50 ( )	D 90	ABSOLUTE LETHAL DOSE
PCR 4099 (racemate)	1318	1615 (1448–1747)	1979	2000
SR 25989 A	1259	1702 (1443–1797)	2299	2000
SR 25990 A	3053	4316 (3569–5705)	6137	5000
SR 25990 C	2257	2591 (2372–2805)	2974	4000
SR 25990 D	2634	4268 (3381–6012)	6914	5000

( ) = confidence interval

The pharmacological study just presented has demonstrated the interesting inhibitory properties towards platelet aggregation of the compound Id and the absence of any activity of its isomer I1.

The medicine of the invention can be made available for oral administration in the form of tablets, sugar-coated tablets, capsules, drops, granules or a syrup. It can also be made available for rectal administration in the form of suppositories or for parenteral administration in the form of an injectable solution.

Each unit dose contains advantageously from 0.001 g to 0.100 g of the derivative of the invention, and the daily doses to be administered may vary from 0.001 g to 0.500 g of active ingredient depending on the age of the patient and the severity of the disorder to be treated. Some pharmaceutical formulations of the medicine of the invention will be given below as non-restrictive examples.

(1) Tablets

Active ingredient: 0.010 g

Excipient: lactose, powdered sugar, rice-starch, alginic acid, magnesium stearate

(2) Sugar-coated tablets

Active ingredient: 0.005 g

Excipient: magnesium stearate, maize starch, gum arabic, shellac, white sugar, glucose, white wax, carnauba wax, paraffin, cochineal.

(3) Capsules

Active ingredient: 0.025 g

Excipient: magnesium stearate, maize starch, lactose.

(4) Injectable solution

Active ingredient: 0.050 g

Isotonic saline q.s.p. 3 ml

(5) Suppositories

Active ingredient: 0.030 g

Semi-synthetic triglycerides q.s.p. 1 suppository.

On account of its interesting inhibitory properties towards platelet aggregation and its interference in the mechanism of formation of arterial and venous thromboses, the medicine of the invention can be usefully administered in the treatment and prevention of platelet disorders due to extracoporeal blood circuits or the consequence of complications in atheroma.

We claim:

1. Dextro-rotatory isomer of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate substantially separated from the levo-rotatory isomer and its pharmaceutically acceptable salts.

2. Hydrochloride of the dextro-rotatory isomer of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate substantially separated from the levo-rotatory isomer.

3. Hydrogen sulfate of the dextro-rotatory isomer of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate substantially separated from the levo-rotatory isomer.

4. Hydrobromide of the dextro-rotatory isomer of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate substantially separated from the levo-rotatory isomer.

5. Taurocholate of the dextro-rotatory isomer of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thieno pyridyl) (2-chlorophenyl)-acetate substantially separated from the levo-rotatory isomer.

6. Pharmaceutical composition which comprises an effective platelet aggregation inhibiting amount of a compound of claim 1 and a pharmaceutically acceptable carrier.

7. Composition according to claim 6, comprising unit doses containing from 0.001 g to 0.100 g of active ingredient.

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